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Whole chromosome painting of B chromosomes of the red-eye tetra *Moenkhausia sanctaefilomenae* (Teleostei, Characidae)

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Abstract

B chromosomes are dispensable genomic elements found in different groups of animals and plants. In the present study, a whole chromosome probe was generated from a specific heterochromatic B chromosome occurring in cells of the characidae fish *Moenkhausia sanctaefilomenae* (Steindachner, 1907). The chromosome painting probes were used in fluorescence *in situ* hybridization (FISH) experiments for the assessment of metaphase chromosomes obtained from individuals from three populations of *M. sanctaefilomenae*. The results revealed that DNA sequences were shared between a specific B chromosome and many chromosomes of the A complement in all populations analyzed, suggesting a possible intra-specific origin of these B chromosomes. However, no hybridization signals were observed in other B chromosomes found in the same individuals, implying a possible independent origin of B chromosome variants in this species. FISH experiments using 18S rDNA probes revealed the presence of non-active ribosomal genes in some B chromosomes and in some chromosomes of the A complement, suggesting that at least two types of B chromosomes had an independent origin. The role of heterochromatic segments and ribosomal sequences in the origin of B chromosomes were discussed.

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Keywords
Chromosomal evolution, Chromosome microdissection, Chromosome painting, Genome organization, Repetitive DNA

Introduction

Supernumerary or B chromosomes are defined as nonhomologous extra genomic elements that actively recombine with chromosomes of the A complement (Jones and Rees 1982, Camacho et al. 2000). They are also characterized by their peculiar evolutionary mode in the genome of carrier species (Jones and Rees 1982, Beukeboom 1994). Additionally, B chromosomes represent dispensable genetic material and can persist in natural populations without being eliminated by natural selection (Carvalho et al. 2008). Studies on the biology of supernumerary chromosomes include analyses of their distribution among species, structure and origin, inheritance, population dynamics and evolution. They further comprise the integration of B chromosomes with chromosomes of the A complement (Camacho et al. 2000, Bugrov et al. 2007). The occurrence of B chromosomes in fish is uncommon and has been described in about 5% of species already karyotyped (Oliveira et al. 2009, Arai 2011).

The red-eye tetra *M. sanctaefilomenae* (Steindachner, 1907) provides a good model for studying B chromosomes in fishes. This species possesses (i) from 0 to 8 B chromosomes (with numbers varying intra- and inter-individually); (ii) B chromosomes with a remarkable polymorphism, morphology and structure (as evidenced by C-banding, with either euchromatic or partially or fully heterochromatic Bs); (iii) putative sex-related B chromosomes (Foresti et al. 1989, Portela-Castro et al. 2001, Hashimoto et al. 2012). However, considering the scarce data about the molecular structure, homology among different B chromosomes and inferences about the origin of *M. sanctaefilomenae*, the aim of this work was to apply physical mapping of ribosomal DNA, microdissection and chromosome painting techniques to address these questions.

Material and methods

We collected 30 individuals (21 males and 9 females) of *M. sanctaefilomenae* from Araquá stream 22°44.83'S and 48°28.5'W (DDM), 19 individuals (10 males and 9 females) from Mané-Teixeira stream 22°45.78'S and 48°15.71'W (DDM) and 6 individuals (3 males and 3 females) from Olaria stream 21°9.18'S and 50°3.06'W (DDM), all tributaries of the Tietê River, São Paulo, Brazil. Vouchers were deposited in the fish collection of Laboratório de Biologia e Genética de Peixes, UNESP, Botucatu (LBP 18986/18987/18988, LBP18983 and LBP18982, respectively). The samples were collected in accordance with the Brazilian environmental protection legislation (collection permission MMA/IBAMA/SISBIO - No 3245). The procedures for sampling, maintenance and analysis of the specimens were performed in compliance with the

Brazilian College of Animal Experimentation (COBEA) and approved by Bioscience Institute/UNESP Ethics Committee on use of animals (CEUA) (protocol 405).

The mitotic chromosomes were obtained from kidney and gill tissues using the technique described by Foresti et al. (1993). Triple fluorochrome staining with CMA_3/DA/DAPI followed the procedures described by Schweizer (1980). For DNA extraction, we used the Wizard Genomic DNA Purification Kit (Promega) following the manufacturer's instructions. Probes of 18S rDNA were obtained by PCR (Polymerase Chain Reaction) from the total DNA of *M. sanctaefilomenae* from the Araquá stream using the primers 18S F (5'CCG CTT TGG TGA CTC TTG AT 3') and 18S R (5'CCG AGG ACC TCA CTA AAC CA 3') (White et al. 1990).

Microdissection of the B chromosome which are easily identified after GC-specific triple fluorochrome staining (CMA_3/DA/DAPI), was performed using an Eppendorf Transfer Man NK2 micromanipulator coupled to a Zeiss Axiovert 100 microscope. Ten B chromosomes, previously identified as heterochromatic B chromosomes using the CMA_3/DA/DAPI staining protocol, were microdissected and transferred into a microcentrifuge tube containing a DOP-PCR mix solution. The probes, here referred to as MsB, were obtained by PCR (Polymerase Chain Reaction) using a DOP primers according to Telenius et al. (1992) and the technique described by Diniz et al. (2008).

For FISH experiments, the chromosomes were treated according to the procedures described by Pinkel et al. (1986). The probes were labeled by PCR with biotin-16-dUTP (Roche Applied Science) and the signal was detected with avidin-FITC (Roche Applied Science). Or else, they were labeled with Digoxigenin-11-dUTP (Roche Applied Science) and the signal was detected with anti-digoxigenin-rhodamine (Roche Applied Science). The images were captured with a digital camera (Olympus DP70) attached to an Olympus BX61 epifluorescent photomicroscope. Image treatment, including karyotype mounting and optimization of brightness and contrast, was performed using the Adobe Photoshop CS2 program.

Results

Hybridization with the MsB probe on metaphase chromosomes of individuals of the Araquá stream resulted in the complete painting of one B chromosome (Fig. 1a), present in 17% of all specimens analyzed. Additionally, fluorescent signals were observed in the pericentromeric region of 16 chromosome pairs in the A complement. Nevertheless, in some cases a single chromosome of a pair was stained as observed in pair 1 (Fig. 1a). FISH experiments with 18S rDNA probes showed positive signals in pairs 7, 12, 15 and 17 of the A complement and in one B chromosome (Fig. 1a), present in 100% of all specimens analyzed.

Individuals from the Mané Teixeira stream showed hybridization signals in the pericentromeric region of 12 chromosome pairs in the A complement and hybridization signals in only one of the homologous of pairs 15 and 22 with the MsB probe. Signals with the 18S rDNA probe were identified in pairs 7, 10, 14, 15 and 17 (Fig. 1b).

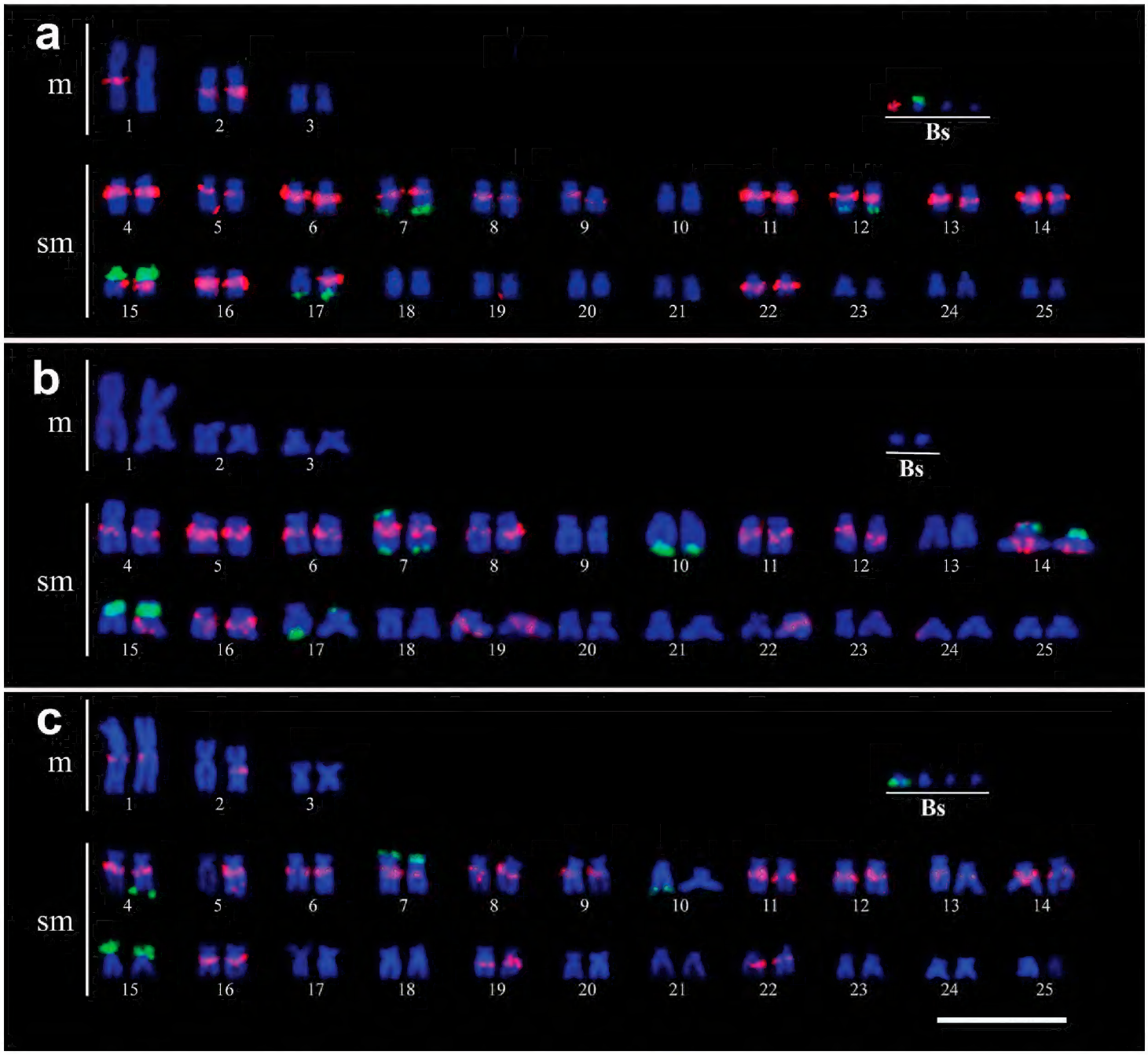

Figure 1. Karyotypes of *Moenkhausia sanctaefilomenae* from the Araquá (**a**), Mané Teixeira (**b**) and Olaria (**c**) streams, arranged from chromosomes after double-FISH. Chromosome painting with MsB-probe (red) and 18S rDNA (green). Bar =10 μm.

In pair 7, one chromosome occured with positive signals in the terminal position of both short and long arms. In pair 17 one chromosome appeared with positive signals in the terminal position of the long arm and the putative homologous with positive signals in the terminal position of the short arm (Fig. 1b). In this population, the MsB and 18S probes did not hybridize with the B chromosomes (Fig. 1b).

Hybridization with the MsB probe in the individuals collected in the Olaria stream showed signals in the pericentromeric region of chromosome pair 15 in the A complement. Notwithstanding, in some cases, a single chromosome of a pair was stained, as observed in pairs 2 and 5 in (Fig. 1c). No hybridization signal was observed in the B chromosomes. FISH with 18S rDNA probes showed positive signals in pairs 4, 7, 10 and 15, but only one of the chromosomes of pairs 4 and 10 were stained (Fig. 1c). B chromosome with 18S sequences were observed in 100% of all specimens analyzed (Fig. 1c).

Discussion

The B chromosomes observed in the populations of *M. sanctaefilomenae* were all very small and characterized as B microchromosomes by Foresti et al. (1989). The occurrence of inter- and intra-individual variation was observed in relation to the number of B chromosomes in the cells of individuals carrying as many as six Bs. Such variation is with accordance with the study by Foresti et al. (1989), who also examined a population of the Tietê River Basin. On the other hand, the individuals from the Paraná River studied by Portela-Castro et al. (2001) showed karyotype differences due to the presence of 0-2 B chromosomes, which were reported to occur only in males. Study by Hashimoto et al. (2012) also showed an inter- and intra-individual variation of the number of B chromosomes in a population from the Batalha River, another tributary of the Tietê River, with individuals carrying up to eight B microchromosomes with a modal number between 2 and 3. All these results showed that the variation in number of B chromosomes in *M. sanctaefilomenae* is very high and that different populations have different modal number of B chromosomes.

Our results of chromosome painting with the DNA probes obtained from the B heterochromatic chromosome from the Araquá Stream population (here referred to as MsB) showed that specific hybridization signals were observed in the pericentromeric region of many chromosomes of the A complement and in only one type of B chromosome. In *M. sanctaefilomenae,* those pericentromeric regions are heterochromatic (Foresti et al. 1989; present study not showed), the hybridization signals found in most chromosomes of the A complement imply that the microdissected B chromosome may have originated from the heterochromatic region of chromosomes of the A complement, as was also shown by Teruel et al. (2009). This finding suggests an intra-specific origin for this B chromosome. Interestingly, putative homologues of A chromosomes inside populations and in different populations have a slightly different pattern of staining with the MsB probe (Fig. 1). This may be caused by small differences involving the MsB sequences in the A chromosomes occur.

The presence of B chromosomes with no signals of hybridization with the specific B chromosome probe pointed to an independent origin of these elements. Alternatively, it is possible to suppose that: (i) in some B chromosomes, the number of the MsB sequences were too small to be detectable by the technique used here; (ii) the loss of some specific chromosome segments during the dynamic process of modification occurred in the independent evolution of different B chromosome lineages; (iii) the B chromosomes that did not stain with the MsB probe may represent B chromosomes derived from different heterochromatic regions; and (iv) the B chromosomes that did not stain with the MsB probe may represent chromosomes recently originated from euchromatic segments.

Sharing of repetitive DNA sequences between B chromosomes and the elements of the A complement is considered a common feature in the chromosomes of different organisms, such as in the mammals *Vulpes vulpes* (Linnaeus, 1758) (Yang et al. 1999) and *Apodemus peninsulae* (Thomas, 1907) (Rubtsov et al. 2004) and in the insects

Podisma sapporensis Shiraki, 1910 (Bugrov et al. 2004), *Podisma kanoi* Storozhenko, 1994 (Bugrov et al. 2007) and *Locusta migratoria* Linnaeus, 1758 (Teruel et al. 2009). Among fish, we also have some reports of sequence sharing between A and B complements, as in the case of *Prochilodus lineatus* (Valenciennes, 1836) in, whose SATHI satellite DNA is shared by both B chromosomes and components of the A set (Jesus et al. 2003; Artoni et al. 2006). However, not all heterochromatic segments in *Prochilodus lineatus,* are composed of SATHI satellite DNA, indicating that other families of repetitive DNA could participate in the structure of the chromosomes in this species. The intra-specific origin of B chromosomes of *Astyanax scabripinnis* (Jenyns, 1842) was also corroborated by Vicari et al. (2011), with the use of chromosome painting.

FISH with 18S probes revealed high variability in the *M. sanctaefilomenae* samples studied here, involving seven chromosome pairs (Fig. 1). However, the number of active NORs is always three: one chromosome pair (pair 15) and a single chromosome of a pair, as observed by Foresti et al. (1989) for this species. These observations are similar with the results obtained for *Triportheus venezuelensis* Malabarba, 2004 by Nirchio et al. (2007), in which the sequences of 18S rDNA were distributed in nine pairs of chromosomes, but the number of chromosomes with active NORs always remained lower. Studies conductd by Daniel et al. (2012) and Silva et al. (2013) detected up to eight sites of 18S rDNA genes in populations of *Astyanax bockmanni* Vari & Castro, 2007, reinforcing the idea that in this species, sites and location of 18S rDNA are usually multiple and variable.

The presence of ribosomal sites in B chromosomes of *M. sanctaefilomenae* suggests that these chromosomes had 18S sequences. Nevertheless, as they are not silver-stained, they may not correspond to active NORs (Foresti et al. 1989, Portela- Castro et al. 2001, Dantas et al. 2007). On the other hand, in fish from the Olaria Stream, ribosomal sites were observed in one B chromosome after silver staining (data not shown here). Mitchell-McGrath and Helgeson (1998), studying hybrids of *Solanum brevidens* Phil. and *Solanum tuberosum* L. and Houben et al. (1997), studying the genus *Brachycome* Cass. suggest that NOR sites are prone to chromosome breakage in plants supporting the hypothesis that B chromosomes could be generated by chromosome fragments coming from these regions. The present results reinforce the hypothesis that chromosome rearrangements involving NOR-bearing chromosomes may be related to the origin of B chromosomes.

According to Lim and Simmons (1994) and Dimitri et al. (1997), the accumulation of repetitive DNA sequences, including transposable elements in specific areas of the chromosomes can render such sites prone to chromosomal rearrangements. Additionally, the molecular structure analysis shows that these B chromosomes may be subject to gene silencing, accumulation of repetitive DNA and also to heterochromatinization processes (Leach et al. 2004). Therefore, the presence of large heterochromatic segments in the A and in some B chromosomes of *M. sanctaefilomenae*, and the reduced number of active ribosomal genes in these chromosomes may corroborate the above hypothesis about the gene silencing due to their close relationship with heterochromatin.

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References

Arai R (2011) Fishes Karyotypes: A Check List. Springer, Tokyo Berlin Heidelberg New York, 347 pp. doi: 10.1007/978-4-431-53877-6

Artoni RF, Vicari MR, Endler AL, Cavallaro ZI, Jesus CM, Almeida MC, Moreira-Filho O, Bertollo LAC (2006) Banding pattern of A and B chromosomes of *Prochilodus lineatus* (Characiformes, Prochilodontidae), with comments on B chromosomes evolution. Genetica 127: 277–284. doi: 10.1007/s10709-005-4846-1

Beukeboom LW (1994) Bewildering Bs an impression of the 1st B – chromosome conference. Heredity 73: 328–336. doi:10.1038/hdy.1994.140

Bugrov AG, Karamysheva TV, Rubtsov DN, Andreenkova OV, Rubtsov NB (2004) Comparative FISH analysis of distribution of B chromosome repetitive DNA in A and B chromosomes in two subspecies of *Podisma kanoi* Storozh (Orthoptera, Acrididae). Cytogenetic and Genome Research 106: 284–288. doi: 10.1159/000079300

Bugrov AG, Karamysheva TV, Perepelov EA (2007) DNA content of the B chromosomes in grasshopper *Podisma kanoi* Storozh (Orthoptera, Acrididae). Chromosome Research 15: 315-325. doi: 10.1007/s10577-007-1128-z

Camacho JPM, Sharbel TF, Beukeboom LW (2000) B chromosome evolution. Phil. Trans. R. Soc. Lond. B 355: 163–178. doi: 10.1098/rstb.2000.0556

Carvalho RA, Martins-Santos IC, Dias AL (2008) Review Paper: B chromosomes: an update about their occurrence in freshwater Neotropical fishes (Teleostei). Journal of Fish Biology 72: 1907–1932. doi: 10.1111/j.1095-8649.2008.01835.x

Dantas ESO, Vicari MR, Souza IL, Moreira-Filho O, Bertollo LAC, Artoni RF (2007) Cytotaxonomy and karyotype evolution in *Moenkhausia sanctaefilomenae*, 1903 (Teleostei, Characidae). The Nucleus 50(3): 505–518.

Daniel SN, Hashimoto DT, Pansonato-Alves JC, Foresti F, Porto-Foresti F (2012) Cytogenetic characterization of distinct B chromosomes in a population of the fish *Astyanax bockmanni* (Teleostei, Characiformes). Caryologia 65(3): 229–233. doi: 10.1080/00087114.2012.738488

Dimitri P (1997) Constitutive heterochromatin and transposable elements in *Drosophila melanogaster*. Genetica 100: 85–93. doi: 10.1023/A:1018304922862

Diniz D, Laudicina A, Cioffi MB, Bertollo LAC (2008) Microdissection and whole chromosome painting. Improving sex chromosome analysis in Triportheus (Teleostei, Characiformes). Cytogenetic and Genome Research 122: 163–168. doi: 10.1159/000163094

Foresti F, Almeida-Toledo LF, Toledo-Filho SA (1989) Supernumerary chromosome systems, C-banding pattern characterization and multiple nucleolus organizer regions in *Moenkhausia sanctaefilomenae* (Pisces, Characidae). Genetica 79: 107–114. doi: 10.1007/BF00057927

Foresti F, Oliveira C, Almeida-Toledo LF (1993) A method for chromosome preparations from large fish specimens using in vitro short-term treatment with colchicine. Experientia 49. doi: 10.1007/BF01923555

Hashimoto DT, Voltolin TA, Arruda-Paes AND, Foresti F, Porto-Foresti F (2012) Cytogenetic analysis of B chromosomes in one population of the fish *Moenkhausia sanctaefilomenae* (Steindachner, 1907) (Teleostei, Characiformes). Comparative Cytogenetics 6: 141–151. doi: 10.3897/CompCytogen.v6i2.1769

Houben A, Leach CR, Verlin D, Rofe R, Timmis JN (1997) A repetitive DNA sequence common to the different B chromosomes of the genus *Brachycome*. Chromosoma 106: 513–519. doi: 10.1007/PL00007689

Jesus CM, Galetti-Júnior PM, Valentini SR, Moreira-Filho O (2003) Molecular characterization and chromosomal localization of two families of satellite DNA in *Prochilodus lineatus* (Pisces, Prochilodontidae), a species with B chromosomes. Genetica 118: 25–32. doi: 10.1023/A:1022986816648

Jones RN, Rees H (1982) B chromosomes. Academic Press, New York, 266 pp.

Leach CR, Houben A, Timmis JN (2004) The B chromosomes in *Brachycome*. Cytogenetic and Genome Research 106: 199–209. doi: 10.1159/000079288

Lim JK, Simmons MJ (1994) Gross chromosome rearrangements mediated by transposable elements in *Drosophila melanogaster*. BioEssays 16: 269–275. doi: 10.1002/bies.950160410

Mitchell-McGrath, Helgeson JP (1998) Differential behavior of *Solanum brevidens* ribosomal DNA loci in a somatic hybrid and its progeny with potato. Genome 41: 435–439. doi: 10.1139/g98-039

Nirchio M, Oliveira C, Ferreira IA, Granado A, Ron E (2007) Extensive polymorphism and chromosomal characteristics of ribosomal DNA in the characid fish *Triportheus venezuelensis* (Characiformes, Characidae). Genetics and Molecular Biology 30: 25–30. doi: 10.1590/S1415-47572007000100007

Oliveira C, Foresti F, Hilsdorf AWS (2009) Genetics of neotropical fish: from chromosomes to populations. Fish Physiology and Biochemistry 35: 81–100. doi: 10.1007/s10695-008-9250-1

Pinkel D, Straume T, Gray JW (1986) Cytogenetic analysis using quantitative high-sensitivity, fluorescence hybridization. Procedures of the National Academy of Sciences of the United States of America 83: 2934–2938. doi: 10.1073/pnas.83.9.2934

Portela-Castro ALB, Júlio Jr. HF, Nishiyama PB (2001) New occurrence of microchromosomes B in *Moenkhausia sanctaefilomenae* (Pisces, Characidae) from the Paraná River of Brazil: analysis of the synaptonemal complex. Genetica 110: 277–283. doi: 10.1023/A:1012742717240

Rubtsov NB, Karamysheva TV, Andreenkova OV (2004) Comparative analysis of micro and macro B chromosomes in the Korean Field mouse *Apodemus peninsulae* (Rodentia, Murinae) performed by chromosome microdissection and FISH. Cytogenetic and Genome Research 106: 289–294. doi: 10.1159/000079301

Schweizer D (1980) Simultaneous fluorescent staining of R bands and specific heterochromatic regions (DA-DAPI bands) in human chromosomes. Cytogenetics and Cell Genetics 27: 190–193. doi: 10.1159/000131482

Silva DMZA, Pansonato-Alves JC, Utsonomia R, Daniel SN, Hashimoto DT, Oliveira C, Porto-Foresti F, Foresti F (2013) Chromosomal organization of repetitive DNA sequences in *Astyanax bockmanni* (Teleostei, Characiformes): dispersive location, association and co-localization in the genome. Genetica 141: 329–336. doi: 10.1007/s10709-013-9732-7

Telenius H, Carter NP, Bebb CE, Nordenskjold M, Ponder BA, Tunnacliffe A (1992) Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. Genomics 13: 718–25. doi: 10.1016/0888-7543(92)90147-K

Teruel M, Cabrero J, Montiel EE, Acosta MJ, Sánchez A, Camacho JPM (2009) Microdissection and chromosome painting of X and B chromosomes in *Locusta migratoria*. Chromosome Research 17: 11–18. doi: 10.1007/s10577-008-9001-2

Vicari MR, Pistune HFM, Castro JP, Almeida MC, Bertollo LAC, Moreira-Filho O, Camacho JPM, Artoni RF (2011) New insights on the origin of B chromosomes in *Astyanax scabripinnis* obtained by chromosome painting and FISH. Genetica 139: 1073–1081. doi: 10.1007/s10709-011-9611-z

White TJ, Bruns T, Lee S, Taylor T (1990) Amplification and direct sequencing of fungal ribosomal RNA for phylogenetics. PCR Protocols: A Guide to Methods and Aplications. Academic Press Inc, London/New York, 312–315. doi: 10.1016/b978-0-12-372180-8.50042-1

Yang F, O`Brien PCM, Milne BS (1999) A complete comparative chromosome map for the dog, red, fox, and human and its integration with canine genetic maps. Genomics 62: 189–202. doi: 10.1006/geno.1999.5989